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ORIGINAL ARTICLE

Retinol and Alpha-tocopherol in the Colostrum of Lactating Tunisian Women Delivering Prematurely: Associations with Maternal Characteristics

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Key Words
colostrum;
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Background: This study aims to assess vitamin A and E concentrations in the premature colostrum of lactating Tunisian women and to identify maternal characteristics that may affect these concentrations.
Methods: Human colostrum was obtained from 105 mothers who gave birth prematurely in the Centre for Maternity and Neonatology of Tunis (Tunisia). Retinol and alpha-tocopherol were analyzed in the colostrum and in plasma by high-performance liquid chromatography.
Results: Retinol and alpha-tocopherol concentrations were 57.5 ± 50.1 μg/dL and 1222 ± 772 μg/dL in the colostrum, respectively, and 51.7 ± 20.0 μg/dL and 1351 ± 772 μg/dL in plasma, respectively. Concentrations of each vitamin in the colostrum were positively correlated with their respective concentrations in plasma ($r = 0.415$, $p = 0.001$ for retinol and $r = 0.392$, $p = 0.003$ for alpha-tocopherol). In multivariate analysis, colostrum vitamin A was associated with plasma vitamin A and preeclampsia, while colostrum vitamin E was associated with plasma vitamin E, gestational age, and preeclampsia.
Conclusion: In Tunisian women, colostrum vitamin A and E levels are close to the average values reported in the literature. The levels are too low to cover the needs of very low birth weight (VLBW) infants, particularly in women with plasma vitamin deficiencies, preeclampsia, or very premature delivery. Given the undeniable beneficial effects of human colostrum,

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whenever feasible, VLBW infants should be fed colostrum. Infant vitamin A and E requirements should be met by milk fortification or supplementation.

1. Introduction

Retinol and alpha-tocopherol are the most important forms of vitamins A and E. These fat-soluble vitamins are vital nutrients for the growing newborn.[1] Very low birth weight (VLBW) infants usually have low plasma and store levels of vitamin A and E.[2,3] Given that they are exposed to a high risk of infection and oxygen exposure, VLBW infants should receive ample doses of these vitamins to stimulate their immune systems and protect them from oxygen toxicity. Human milk is considered the ideal feeding choice for preterm infants,[4] however, it does not contain enough fat-soluble vitamins to meet the needs of VLBW infants.[5–7] Thus, recommendations have been made to supply these infants with fat-soluble vitamins from preterm formula or parental nutrition.[8,9] The quality of breast milk and its vitamin content may vary according to lactation stage. Colostrum, the milk secreted in the first few days following delivery, contains a larger percentage of fat-soluble vitamins than mature milk.[10–13] Breast milk composition also varies according to the term of birth. Preterm milk may be more apt to meet the nutritional requirements of premature infants than term milk.[14,15] The nutrition of preterm infants fed milk from their own mothers result in better growth and nutritional status, including levels of vitamins A and E, compared with nutrition observed in infants fed formula.[16,17] Finally, breast milk composition may be affected by maternal characteristics, such as age, parity, dietary intake, and perhaps gestational diseases.[18–20] To test how the premature colostrum covers the needs of vitamins A and E in VLBW infants, we assessed retinol and alpha-tocopherol in colostrum from lactating women who gave birth prematurely. We also tested the effects of some maternal characteristics that may influence colostrum vitamin A and E content.

2. Methods

2.1. Participants and sample collection

The study included 105 Tunisian lactating women who delivered prematurely (from 28 weeks to 37 weeks of gestation). The women included were mothers of VLBW infants (birth weight $<$ 1500 g and gestational age $<$ 37 weeks) who were hospitalized in the service of Neonatology at the Centre of Maternity and Neonatology of Tunis, Tunisia. The Centre is an inner-city tertiary-care hospital that caters mainly to the needs of a socioeconomically compromised population. Lactating mothers with chronic and acute diseases and those using supplements containing vitamin A or E during pregnancy were excluded. Fresh human colostrum was obtained from lactating mothers between the 2^{nd} day and 7^{th} day postpartum. The mothers expressed one breast in total with a manual breast pump and the colostrum was collected in a sterile glass vial. One milliliter of mixed colostrum was recuperated for the needs of the study, with the remaining portion fed to the infant (whenever possible). In parallel, fasting blood samples from 57 women were collected into tubes containing ethylenediaminetetraacetic acid. Samples were protected from light and transported on ice to the laboratory within 2 hours of collection. Blood samples were centrifuged at 1500*g*, and plasma and colostrum samples stored at -80°C until analysis (within 6 months). Relevant information was collected from the mothers and their medical records, including maternal age, medical and obstetrical history, course of current pregnancy and term, and mode of delivery. Pre-eclampsia and gestational diabetes are defined according to the American College of Obstetricians and Gynecologists criteria.[21,22] All included mothers had been consuming habitual Tunisian food during pregnancy and none had adopted special diets or had received vitamin A or E supplements. The study protocol was approved by the Ethics Committee of the Maternity Center and informed consent was obtained from each woman.

2.2. Analytical methods

Retinol and alpha-tocopherol were assessed using reverse-phase high-performance liquid chromatography to analyze the colostrum and plasma according to the method described by Driskell et al.[23] Colostrum samples were saponified in ethanolic potassium hydroxide. Colostrum or plasma samples were deproteinized in the presence of ethanol containing butylated hydroxytoluene as an antioxidant and retinol acetate as an internal standard. After extraction with hexane and evaporation under nitrogen, residues were dissolved in ethanol and injected onto a C18 reverse-phase column (Shimpack ODS-M, Shimadzu, Kyoto, Japan). The mobile phase consisted of a methanol gradient (Merck, Darmstadt, Germany) at a flow rate of 1.5 mL/min, and vitamins were detected at 290 nm. The sensitivity was 5 μg/dL for retinol and 50 μg/dL for alpha-tocopherol. The long-term (n = 30) imprecisions (CVs) were 6.1% and 5.6% at concentrations of 47 μg/dL and 360 μg/dL and 5.8% and 5.1% at concentrations of 1145 μg/dL and 1445 μg/dL for retinol and alpha-tocopherol, respectively.

2.3. Statistical analysis

Statistical computations were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The data of continuous variables were examined for normality using the Kolmogrov-Smirnov test. Continuous variables were compared using independent-samples *t* tests. Relationships between continuous variables were tested using Pearson's correlation. To identify independent predictors for vitamins A and E in colostrum, backward multiple logistic regression models with $p > 0.10$ as elimination criteria were applied with retinol or alpha-tocopherol concentrations as the response variables. The independent variables were respective plasma vitamin concentrations, maternal age, gestational age, multiple pregnancy, cesarean section, preeclampsia, and gestational diabetes. Goodness-of-fit for logistic models was satisfactory. A *p* value < 0.05 based on a two-sided calculation was considered significant.

3. Results

Maternal age varied from 17 years to 42 years (31.4 $\pm$ 4.9 years), and gestational age varied from 27 weeks to 37 weeks (30.4 $\pm$ 2.4). The study population showed a high rate of twin pregnancies (35.2%), preeclampsia (40%), and gestational diabetes (12.4%). The retinol and alpha-tocopherol concentrations in colostrum and plasma are shown in Figure 1. No significant correlation was observed between the colostrum concentrations of the two vitamins, however, each colostrum vitamin concentration was related to its respective plasma concentration ($r = 0.415$, $p = 0.001$ for retinol and $r = 0.392$, $p = 0.003$ for alpha-tocopherol) (Figure 2). In univariate analysis, both the retinol and alpha-tocopherol concentrations in colostrum were significantly lower in mothers < 35 years of age and in those with preeclampsia. The colostrum alpha-tocopherol concentrations were higher in women with twin pregnancies. No differences were observed according to gestational age, mode of delivery, or occurrence of gestational diabetes (Table 1). In multivariate analysis, colostrum retinol was related to plasma retinol and preeclampsia. Colostrum alpha-tocopherol was related to plasma alpha-tocopherol, gestational age, and preeclampsia (Table 2).

4. Discussion

The colostrum retinol and alpha-tocopherol concentrations in these Tunisian women were close to the average concentrations reported worldwide.[7,10–13,16,24–30] The retinol concentrations were close to those reported in different groups of Brazilian lactating women[29,30] and lower than

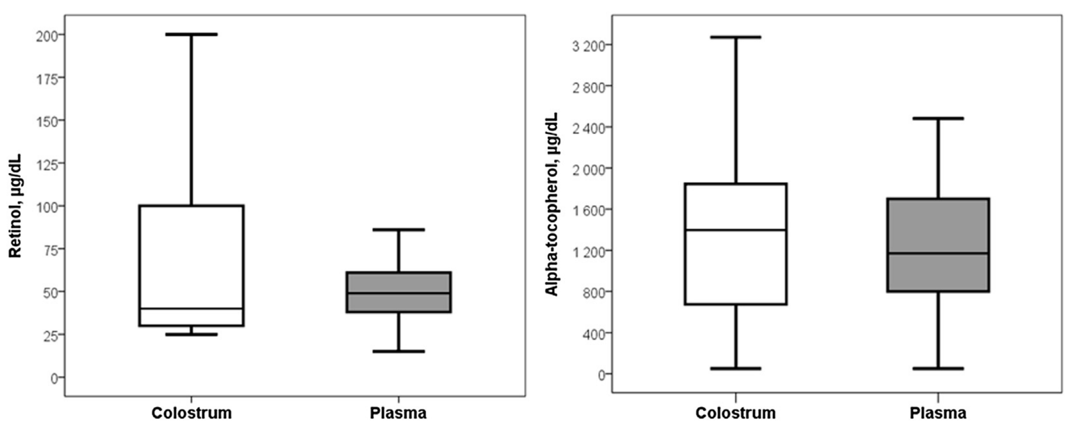

Figure 1 Box plot distribution of the retinol and alpha-tocopherol concentrations in colostrum ($n = 105$) and plasma ($n = 57$) in lactating Tunisian women. * The bottom and top of the box represent the first and third quartiles (IQR) and the band inside the box represents the median. The ends of the whiskers represent the lowest and highest values still within 1.5 IQR. Outlier values are not displayed. IQR = inter-quartile range.

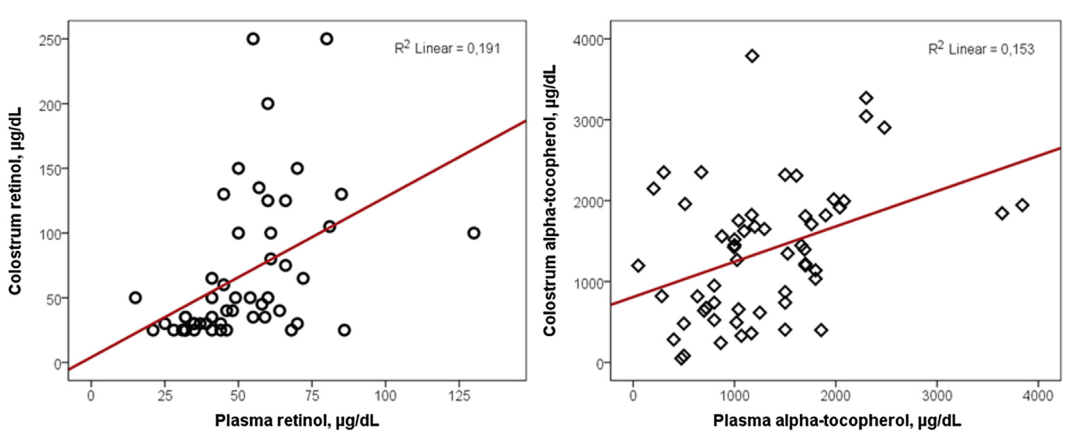

Figure 2 Correlations between colostrum vitamin A and E concentrations with their respective plasma concentrations in lactating Tunisian women ($n = 57$).

Table 1 Retinol and alpha-tocopherol concentrations in preterm colostrum according to selected maternal characteristics.

	n	Retinol (μg/dL)	Alpha-tocopherol (μg/dL)
Maternal age (y)			
17–35	75	62.3 ± 55.1	1315 ± 644
35–42	30	44.3 ± 32.8	903 ± 1009*
Parity			
Nulliparous	26	67.1 ± 50.5	1413 ± 709
Multiparous	79	54.6 ± 48.1	1163 ± 793
Term of birth (wks)			
27–32	77	60.5 ± 54.5	1190 ± 835
32–37	28	49.1 ± 34.6	1312 ± 895
Twin pregnancy			
No	68	54.6 ± 47.1	1108 ± 731
Yes	37	62.9 ± 55.5	1433 ± 812*
Preeclampsia			
No	63	66.5 ± 56.5	1322 ± 860
Yes	42	43.8 ± 34.9*	1069 ± 595*
Gestational diabetes			
No	92	58.3 ± 50.5	1229 ± 809
Yes	13	53.9 ± 50.8	1228 ± 472

Data are presented as mean ± SD.
*$p < 0.05$.
SD = standard deviation.

those of Canadian,[24] German,[26] Turkish,[28] Japanese[11], and Chinese[12] lactating women, but higher than those of lactating women from Poland[13] and Bangladesh.[25] The colostrum alpha-tocopherol concentrations in Tunisian women were close to those of lactating women from Brazil,[29,30] Poland,[7] and Turkey,[28] but were about the half of those reported in lactating women from Germany[26] and Spain[27] (Table 3).[7,10–13,16,21,24–26,28–30] In accordance with the literature, this study revealed a large inter-subject variation in the concentration of the two vitamins, especially vitamin A. The vast inter-study variation could be related to differences in lifestyles, nutritional intake, food fortification, and supplement use. Some methodological factors, such as the day of collection or time elapsed from previous breastfeeding and the previous meal, may contribute to this variability. Finally, analytical differences between studies are also significant sources of variation. The methods used for vitamins hydrolysis (saponification) and extraction may affect vitamin levels in milk, particularly for vitamin A, which is mostly present in the colostrum as retinyl esters.

Strong and convincing evidence indicates that feeding human colostrum to preterm infants has beneficial effects for short- and long-term outcomes.[4,31,32] Given that VLBW infants are at high risk for poor outcomes, it is assumed that these infants may gain great benefits from feeding on colostrum. This study was conducted to verify whether the colostrum of mothers delivering prematurely covers the needs of VLBW infants with regard to vitamins A and E. In practice, the nutritional requirements of VLBW infants are usually met by combined parenteral/enteral regimens. Whenever feasible and as soon as possible, enteral feeding with small amounts of milk/formula is started, and the supply of milk is gradually increased in parallel with the reduction of parenteral fluids. Feeding with human colostrum is preferred and the daily volume of feeding rarely exceeds 100 mL during the first few days of life. Considering the concentrations observed in these women, 100 mL of colostrum provides an average dose of 50 μg of vitamin A and of 1500 μg of vitamin E. These doses are markedly lower than the currently recommended doses of 300–800 μg retinol equivalent/kg and 2000–8000 μg alpha-tocopherol equivalent/kg.[8,9] As a result, additional doses of vitamins A and E should be supplied to VLBW infants using vitamin-fortified milk, vitamin-rich formula, or parenteral nutrition.

To identify lactating women at risk for vitamin deficiencies, we tested the effects of some maternal characteristics on breast milk vitamin status. Our data showed that the concentration of each vitamin in colostrum is positively correlated with its respective concentration in plasma. Such associations have been reported in previous studies[16,33] and have been confirmed following vitamin supplementation.[17,34] These findings emphasize the importance of maternal status as a determinant of the milk vitamin content. The mechanisms by which vitamins are transported to the mammary glands are not well understood. Schweigert and Eisele[35] studied the transport of fat-soluble vitamins and lipids in cows, suggesting that vitamins A and E might be transferred to the mammary glands by a secretory cell transport system specific to retinol binding protein and low-density lipoproteins, respectively. Other studies have found no correlation of milk vitamins A and E with their plasma concentration, suggesting the existence of alternative transport mechanisms to the mammary glands for these vitamins.[19,25,36] This study showed higher

Table 2 Backward multiple logistic regression modeling using colostrum retinol or colostrum alpha-tocopherol as the dependent variable ($n = 57$).*

	Colostrum retinol		Colostrum alpha-tocopherol	
	Standardized β coefficient	*p*	Standardized β coefficient	*p*
Plasma retinol	0.361	0.007	—	—
Plasma alpha-tocopherol	—	—	0.460	<0.001
Gestational age	−0.154	0.213	0.362	0.003
Preeclampsia	−0.280	0.033	−0.289	0.016

* Variables initially entered in the models are the plasma vitamin concentration (retinol or alpha-tocopherol, respectively), maternal age, parity, term of birth, multiple pregnancy, preeclampsia, and gestational diabetes.

Table 3 Vitamin A and E concentrations in the colostrum of lactating women from different regions of the world.

Study	Place	Sample size	Day(s) post-partum	Retinol (μg/dL)	Alpha-tocopherol (μg/dL)
Chappell et al[24] (1985)	Canada	12	1^{st}–6^{th}	145	1100
Ahmed et al[25] (2004)	Bangladesh	105	2^{nd}	22.6 ± 12.0	918 ± 364
Schweignert et al[26] (2004)	Germany	21	2^{nd}	153 ± 72.5	2197 ± 1337
Macias and Schweigert[10] (2001)	Cuba	21	2^{nd}–3^{rd}	102 ± 56.0	1180 ± 630
Quiles et al[27] (2006)	Spain	15	3^{rd}	—	2455
Sakurai et al[11] (2005)	Japan	6	6^{th}–10^{th}	93.3 ± 28.6	576 ± 25.6
Orhon et al[28] (2009)	Turkey	20	7^{th}	246 ± 20.0	1324 ± 69.0
Garcia et al[16] (2010)	Brazil	37	1^{st}	74.5 ± 54.4	1236 ± 202
de Lira et al[29] (2012)	Brazil	103	1^{st}	62.5 ± 22.9	1124 ± 551
Shi et al[12] (2011)	China	7	3^{rd}–7^{th}	207 ± 146	294 ± 188
Szlagatys-Sidorkiewicz et al[13] (2012)	Poland	49	3^{rd}	12.6 (7.91–15.9)*	886 (552–1200)*
Grilo et al[30] (2013)	Brazil	93	Immediate period	46.8 (33.9–103)*	1148 ± 630
Martysiak-Żurowska et al[7] (2013)	Poland	48	2^{nd}	—	999 ± 445
Present study	Tunisia	105	2^{nd}–7^{th}	57.5 ± 50.1	1222 ± 772

Data are presented as mean ± SD unless otherwise indicated.
SD = standard deviation.
* Median (25^{th}–75^{th} percentile).

vitamin A, but lower vitamin E levels in the colostrum of women delivering very prematurely (< 32 weeks) compared with women delivering between 32 weeks and 37 weeks of gestation. The association remained significant only for vitamin E when adjusted for potential confounding factors. The mechanisms responsible for these differences have not been elucidated, however, these levels remain too low to cover vitamin A and E requirements in premature infants.

The novel findings from the present study constitute the low colostrum vitamin A and E levels found in preeclamptic women. Such findings could be related to preeclampsia-associated vascular abnormalities that may impair the transfer of vitamins to the mammary gland. No data exist on breast milk vitamin A and E levels in women suffering from preeclampsia, however, it was demonstrated that low plasma vitamin A and E levels were associated with a higher risk for preeclampsia.[37,38] Additionally, periconceptional multivitamin supplementation resulted in a reduction of preeclampsia risk.[39] In light of these findings, preeclampsia could be considered to be a risk factor for vitamin A and E deficiencies in lactating women and their breastfed infants. This provides an additional reason for the screening and tight control of preeclampsia in pregnant women.

This study showed that vitamin A and E levels are lower in older and multiparous women and that vitamin E is higher in women with twin pregnancies. These differences were either not significant or lost their significance following multivariate analysis. The literature also revealed contradictory data about the effects of maternal characteristics on retinol and alpha-tocopherol levels in human milk. Further research should be undertaken in larger samples with the standardization of physiological and methodological factors to identify lactating women with deficiencies in these vitamins.

Our study included a relatively large number of pregnant women. The sample size is among the largest extant compared with analogous studies. Vitamins were assessed in parallel in plasma, while plasma was only available in 57 lactating women. However, dietary intake by the mothers and the quantity of vitamins received by the infants were not recorded. Additionally, almost all included women were of low-to-average socioeconomic rank, rendering the findings not generalizable to all Tunisian lactating women.

5. Conclusion

In summary, the vitamin A and E concentrations in colostrum in Tunisian women are close to the average values reported worldwide. Considering the small volume of daily feedings, vitamin A and E content in colostrum is insufficient to cover the needs of VLBW infants. This shortfall is even more marked if the lactating women have plasma vitamin deficiencies, suffer from preeclampsia, or deliver very prematurely. A tight follow-up schedule for pregnant women, with screenings and control of nutritional deficits and preeclampsia, would improve vitamin A and E content in colostrum. Considering the undeniable beneficial effects of human colostrum, whenever possible, VLBW infants should be fed colostrum during the first days of life. Given their low vitamin A and E status and their high risk for poor outcomes, VLBW infant requirements for these vital nutrients should be met by milk fortification or supplementation.

Conflicts of interest

The authors declare no conflicts of interest.

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